ARTICLE 34 AMD'T

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method for the *in vitro* micropropagation and phytofortification of a phytopharmaceutical plant comprising:

a) culturing a sterile explant of said phytopharmaceutical plant on an induction medium comprising at least one plant growth regulator having cytokinin activity, to form regenerated tissue;

b) transferring said regenerated tissue to a basal medium and culturing to form plantlets; and

c) subculturing said plantlets onto a basal medium containing at least one additive of interest, to allow uptake and accumulation of said at least one additive of interest in a bio-available form in said plantlet.

2. The method of claim 1, wherein after said step of culturing (step a)), and prior to said step of transferring (step c)), said regenerated tissue is placed on a basal medium and subcultured to allow optimized formation of regenerated tissue; and

3. The method of claim 1 wherein after said step of transferring (step b)), said plantlet is transferred to a hydroponic environment with a recycling solution containing at least one additive of interest to allow uptake and accumulation of said at least one additive of interest in a bioavailable form within said plantlet or seedling.

4. The method according to any one of claim 1, 2 or 3, wherein in said culturing step, said at least one additive of interest is selected from boron, calcium, chloride, chromium, cobalt, copper, iron, lithium, iodine, magnesium, manganese, molybdenum, nickel, phosphorous, potassium, selenium, silicon, sodium, sulphur, tin, vanadium and zinc.

Sub A1

5. The method of any one of claims 1 to 4, wherein said phytopharmaceutical plant is selected from the group consisting of:

Achillea millefolium

Achyranthes bidentata

ARTICLE 34 AMD'T

Aconitum napellus
Adonis aestivalis
Agastache mexicana
Agrimonia eupatoria
Agathosma betulina
Allium sp
Anchusa officinalis
Anemopsis californica
Angelica dahurica
Angelica polymorpha sinensis (A. sinensis)
Arnica Montana
Ammi visnaga
Arctostaphylos uva-ursi
Asclepias tuberosa
Astragalus membranaceus
Astragalus chinensis
Baphicacanthus cusia
Bixa orellana
Bupleurum falcatum
Brugmansia (Datura) spp.
Campanula rapunculus
Carum roxburgianum
Carum copticum
Cassia tora
Chamaelirium luteum
Chimaphila umbellata
Commiphora africana
Conium maculatum
Crithium maritimum
Datura metel (Datura alba)
Datura inoxia
Dracocephalum moldavica
Echinacea sp.

ARTICLE 34 AMDT

Eclipta alba (E. prostrata)
Ephedra nevadensis
Eriodictyon californicum
Eucommia ulmoides
Eupatorium perfoliatum
Filipendula vulgaris (F. hexapetala)
Gaultheria procumbens
Geum urbanum
Houttuynia cordata
Hydrocotyle asiatica (Centella asiatica)
Hypericum perforatum cv. Anthos
Inula helenium
Jatropha curcas
Leptospermum scoparium
Lespedeza capitata
Ligusticum porteri
Ligustrum lucidum
Lithospermum officinale
Lycium barbarum
Mucuna pruriens
Mandragora officinarum
Origanum dictamnus
Parietaria judaica (P. officinalis)
Phyllanthus emblica
Picrasma excelsa
Piniella ternate
Pogostemon patchouli
Polygonum multiflorum
Porophyllum ruderale ssp. macrocephalum
Prunella vulgaris
Pueraria lobata (P. thunbergiana)
Rauvolfia serpentina
Rivea corymbosa

ARTICLE 34 AMDT

Sanguinaria Canadensis
Satureja douglasii
Schizonepeta tenuifolia
Scutellaria baicalensis
Solanum xanthocarpum (S. surattense)
Sutherlandia frustescens
Tabebuia impetiginosa
Tanacetum parthenium
Tribulus terrestris
Trichosanthes kirilowii
Turnera diffusa
Voacanga africana, and
Withania somnifera

6. The method according to claim 5, wherein said phytopharmaceutical plant is selected from St. John's wort (*Hypericum perforatum* cv. Anthos), Huang-qin (*Scutellaria baicalensis*), *Echinacea sp.* and feverfew (*Tanacetum parthenium*).

7. The method according to any one of claims 1 to 6, wherein said at one plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron (TDZ, *N*-phenyl-*N'*-(1,2,3-thidiazol-yl)urea), benzylaminopurine (BAP), zeatin, CPPU (N-(2-chloro-4pyridyl)-N(-phenyl urea) and 2-*i*-P (N6-(2-isopentenyl) adenine or 6-gamma,gamma-dimethylallylamino purine).

8. The method according to claim 7, wherein said at least one plant growth regulator having cytokinin activity is selected from thidiazuron (TDZ) and benzylaminopurine (BAP).

9. The method according to claim 8, wherein said induction medium comprises from about 0.001 to about 25 $\mu mol \cdot L^{-1}$ of said at least plant growth regulator having cytokinin activity.

10. The method according to claim 8, wherein said sterile explant is maintained on said induction medium from about 1 to about 50 days.

Sub Q3

11. The method according to any one of claims 1 to 10, wherein said explant is selected from the seed, petiole, hypocotyl, stem, cotyledon and leaf.

12. The method according to any one of claims 1 to 4, wherein said phytopharmaceutical plant is St. John's wort.

13. The method according to claim 12, wherein said plant growth regulator having cytokinin activity is thidiazuron.

14. The method according to claim 13, wherein the induction medium comprises thiadiazuron from about 0.001 to about 25 $\mu mol \cdot L^{-1}$.

15. The method according to claim 14, wherein the induction medium comprises thiadiazuron from about 4 to about10 $\mu mol \cdot L^{-1}$.

16. The method according to claim 12, wherein said sterile explant is maintained on said induction medium from about 1 to about 15 days.

17. The method according to claim 16, wherein said sterile explant is maintained on said induction medium from about 8 to about 10 days.

18. The method according to claim 12, wherein said explant is etiolated hypocotyl.

Sub Q4

19. The method according to any one of claims 1 to 4, wherein the phytopharmaceutical plant is *Echinacea sp.*.

20. The method according to claim 19, wherein said plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron and benzylaminopurine.

21. The method according to claim 20, wherein said induction medium comprises from about 0.001 to about 25 $\mu mol \cdot L^{-1}$ of said plant growth regulator having cytokinin activity.

22. The method according to claim 20, wherein said plant growth regulator having cytokinin activity is from about 1.0 to about 15 $\mu mol \cdot L^{-1}$.

23. The method according to claim 19, wherein said sterile explant is maintained on said induction medium from about 1 to about 50 days.

24. The method according to claim 23, wherein said sterile explant is maintained on said induction medium from about 10 to about 35 days.

25. The method according to claim 19, wherein said explant is petiole.

SUB CLAIM

26. The method according to any one of claims 1 to 4, wherein said phytopharmaceutical plant is Huang qin.

27. The method according to claim 26, wherein said plant growth regulator having cytokinin activity is thidiazuron.

28. The method according to claim 27, wherein said induction medium comprises from about 0.001 to about 25 $\mu mol \cdot L^{-1}$ of said plant growth regulator having cytokinin activity.

29. The method according to claim 28, wherein said plant growth regulator having cytokinin activity is from about 1.5 to about 20 $\mu mol \cdot L^{-1}$

30. The method according to claim 26, wherein said sterile explant is maintained on said induction medium from about 1 to about 30 days.

31. The method according to claim 30, wherein said sterile explant is maintained on said induction medium from about 14 to about 20 days.

32. The method according to claim 26, wherein said explant is selected from seeds, hypocotyl and stems.

ARTICLE 34 AMD'T

33. The method according to any one of claims 1 to 4, wherein the phytopharmaceutical plant is feverfew.

34. The method according to claim 33 wherein said plant growth regulator having cytokinin activity is thidiazuron.

35. The method according to claim 34, wherein said induction medium comprises from about 0.001 to about 25 $\mu mol \cdot L^{-1}$ of said plant growth regulator having cytokinin activity.

36. The method according to claim 35, wherein said plant growth regulator having cytokinin activity is from about 2.0 to about 8.0 $\mu mol \cdot L^{-1}$

37. The method according to claim 33, wherein said sterile explant is maintained on said induction medium from about 1 to about 50 days.

38. The method according to claim 37, wherein said sterile explant is maintained on said induction medium from about 20 to about 35 days.

39. The method according to claim 33, wherein the explant is selected from leaf, stem, petiole and hypocotyl.

40. The method according to claim 4, wherein said at least one additive of interest is zinc.

41. A method according to claim 4, wherein said at least one additive of interest is lithium.

42. The method according to claim 4, wherein said at least one additive of interest within said basal medium, is from about 0.001 to about 500 $mg \cdot L^{-1}$.

43. ~~44.~~ The method according to claim 2, wherein, in said transferring step, said regenerated tissue is subcultured for about 1 to about15 days.

ARTICLE 34 AMD'T.

44

45. A method for phytofortification of an *in vitro*-grown phytopharmaceutical plant comprising:

a) culturing a sterile seedling, explant or regenerated tissues to form a plantlet; and

b) subculturing said plantlet onto a basal medium containing at least one additive of interest, to allow uptake and accumulation of said at least one additive of interest in a bio-available form in said plantlet.

45 44

46. The method according to claim 45, wherein, in said step of culturing, said plantlets are produced either:

a) on a sterile explant of said phytopharmaceutical plant grown on an induction medium comprising at least one plant growth regulator having cytokinin activity, or

b) grown from a sterile seed, or

c) seedling in culture.

46 45

47. The method according to claim 46, wherein said at one plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron (TDZ, *N*-phenyl-*N'*-(1,2,3-thidiazol-yl)urea), benzylaminopurine (BAP), zeatin, CPPU (N-(2-chloro-4pyridyl)-N(-phenyl urea) and 2-*i*-P (N6-(2-isopentenyl) adenine or 6-gamma,gamma-dimethylallylamino purine).

47 44

48. A phytopharmaceutical plant prepared by the method of any one claims 1 to 4, or 45 to 47 and comprising an elevated level of said additive of interest when compared to a plant grown in the absence of said additive of interest.

48

49. A method for the *in vitro* micropropagation involving *de novo* shoot formation of non-meristematic tissue of a phytopharmaceutical plant comprising:

a) culturing a sterile explant of said phytopharmaceutical plant on an induction medium comprising one or more plant growth regulators having cytokinin activity, to form regenerated tissue; and

b) transferring said regenerated tissue to a basal medium and culturing to form plantlets.